REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED
03-10-2003	Final Report	01-01-2000 - 30-09-2003

4. TITLE AND SUBTITLE

Elastin: A Stimuli Responsive Bipolymer for Nano-, and Micro-Actuation

5a. CONTRACT NUMBER
N00014-00-1-0183

5b. GRANT NUMBER
N00014-00-1-0183

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
203, Farris Engineering Center, Center for Micro-Engineered Materials, Department of Chemical & Nuclear Engineering, The University of New Mexico, Albuquerque, NM 87131.

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

10. SPONSOR/MONITOR'S ACRONYM(S)
ONR

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AILABILITY STATEMENT
Distribution Unlimited

DISTRIBUTION STATEMENT A
Approved for Public Release
Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

See Enclosed Document

20031017 033

15. SUBJECT TERMS
Stimuli Responsive Polymers, Silica, Actuators, Hybrid Membranes, Controlled Transport Porous Materials, Switchable Surfaces.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Prof. Gabriel P. Lopez
Unclass.	Unclass.	Unclass.	Unlimited	7	19b. TELEPHONE NUMBER (Include area code) (505) 277 4939

Elastin: A Stimuli Responsive Biopolymer for Nano-, and Micro-Actuation

Principal Investigator: Gabriel P. Lopez

GRANT #: N00014-00-1-0183

Abstract

We developed hybrid materials comprising of silica and stimuli responsive polymers (SRPs) to demonstrate the feasibility of SRPs as actuation elements in functional nanostructured materials. We successfully demonstrated that the hybrid membranes comprising of silica and SRPs can function as molecular filters with reversible permeability and good stability. We dispersed these SRPs in a slilica matrix at a molecular level using sol-gel process. The encapsulated SRP molecules to act as nano-valves whose permeability can be controlled by cycling through the lower critical solution temperature (LCST) of the SRPs. Micropatterning studies on hybrid membranes suggest that a wide variety of water-soluble species can be patterned without need for a specific chemisorption or affinity label using localized heating with a UV laser. Developed porous silica nanocomposite materials and modified the surface with SRPs using atom transfer radical polymerization and demonstrated that the size and surface energy of the pores can be externally and reversibly controlled to dynamically modulate the adsorption and transport of molecular species. These have potential applications in the areas of separations, controlled drug delivery, environmental remediation, bioassays, biosensing, tissue engineering.

Using a model system of silica and PNIPAAM we have demonstrated design principles and synthetic methods are applicable to a wide variety of porous structures and polymers (or small molecules) which are reversibly sensitive to different external stimuli such as temperature, light, electricity, pH, solutes, or enzymatic transformations.

FINAL REPORT

GRANT #: N00014-00-1-0183

PRINCIPAL INVESTIGATOR: Prof. Gabriel P. Lopez

INSTITUTION: The University of New Mexico

GRANT TITLE: Elastin: A Stimuli Responsive Biopolymer for Nano-, and Micro-Actuation

AWARD PERIOD: 1 December 1999 – 30 November 2002

OBJECTIVE: To examine the feasibility of elastin like polypeptides (ELP) and poly(N-isopropyl acrylamide) (PNIPAAM) as nanoscopic chemo-mechanical actuators and to understand the physico-biochemical properties of these stimuli-responsive polymers (SRPs)and to demonstrate the feasibility of SRPs as actuation elements in functional nanostructured materials.

APPROACH: SRPs were encapsulated in dense silica matrices using sol-gel processing to prepare smart hybrid nanocomposite membranes. By dispersing the SRPs at a molecular level, the encapsulated SRP molecules to act as nano-valves whose permeability can be controlled by cycling through the lower critical solution temperature (LCST) of the SRPs. Molecular permeability through these hybrid materials was controlled by the extended and contracted conformation of ELPs and PNIPAAM. We exploited the thermo-responsive conformational changes of PNIPAAM chains to reversibly create pores in a silica matrix for a variety of applications which include micropatterning of dye molecules, low molecular weight polymers and peptides labeled with fluorescein.

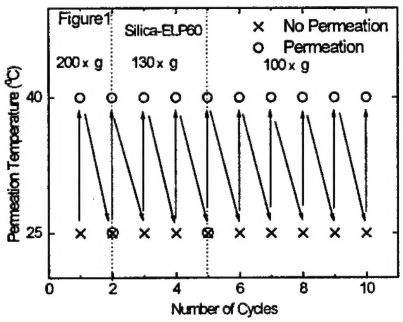
Ordered mesoporous silica microspheres were prepared by a surfactant templating technique using evaporation induced self-assembly. PNIPAAM was grafted into the mesopores of silica matrix using the novel approach of atom transfer radical polymerization (ATRP). In this process, the life time of growing radical on the surface is high (several hours) enabling the preparation of polymers with predefined molar masses, low polydispersity, controlled compositions and functionality. A major advantage of ATRP is that the free radical is confined to the growing polymer chain, thus eliminating the problem of free radicals in solution forming bulk polymer which may in turn clog the pores. Capture and release of fluorescent probes in SRP-grafted silica microspheres were studied by flow cytometry.

Novel temperature sensitive surfaces can also be prepared by grafting SRPs on gold surfaces using ATRP. We have synthesized dense and uniform polymer brushes of PNIPAAM and copolymers of NIPAAM and *N*- tert butyl acrylamide with ATRP on gold surfaces at room temperature. The advantages of ATRP for this purpose include precise and independent control of the thickness, density and composition of the

polymer films. The transitions of these smart surfaces were studied by surface plasmon resonance (SPR), contact angle goniometry and ellipsometric techniques.

ACCOMPLISHMENTS :

Smart Hybrid Membranes: We developed molecular switches as nanoscopic actuators that can control the transport of chemical species by encapsulating ELPs or PNIPAAM in a dense silica gel. Figure 1 illustrates that the hybrid membranes could function as permeability switches upon cycling between 25°C (below LCST) and 40°C (above LCST) with good mechanical stability. Permeation experiments with monodisperse poly(ethyleneglycol) (PEGs)on silica-PNIPAAM and silica-ELP filters demonstrated that these could function as switchable molecular filters (Table 1). Figure 2 demonstrates that change of hydrophilic to hydrophobic state of hybrid membrane by changing the temperature.



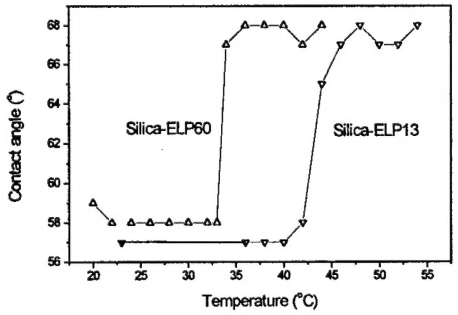


Figure 1. (left) illustrates that the hybrid membranes could function as on/off permeability switches when they cycle between 25°C (below LCST) and 40°C (above LCST).

Figure 2. (right) demonstrates that change of hydrophilic to hydrophobic state of hybrid membrane by changing the temperature.

Table 1. Permeation of poly(ethylene glycol) (PEG) solutions through silica-ELP60 (molecular weight 60 kDa) membranes coated on YM-30 centrifugal filters (Refractive index (R.I) of pure water is 1.3330.

PEG M_w(Da)	Concentration (wt%)	R.I. of feed (± 0.0002)	R.I. of filtrate (± 0.0002)	% Rejection
1000	3.5	1.3370	1.3370	0
2200	2	1.3348	1.3345	0
5000	2	1.3352	1.3328	100
7850	2	1.3355	1.3330	100
9000	3	1.3366	1.3330	100

Micro-patterning of Smart Hybrid Membranes: We utilized the conformational changes of SRPs in a silica matrix for patterning various aqueous molecular species by entrapping them in the pores created by the LCST behavior of the SRPs. This method enabled the patterning of wide variety of water-soluble species without the need for specific chemisorption or affinity labels. This process is reversible; entrapped molecules are released when the substrate is rinsed with water at 40°C. Figure 3 shows patterning of fluorescein on hybrid membranes. Fluorescein molecules are entrapped in the pores of SRP membranes due to localized heating with a UV laser. This process is reversible; entrapped molecules are released when the substrate is rinsed with water at 40°C.

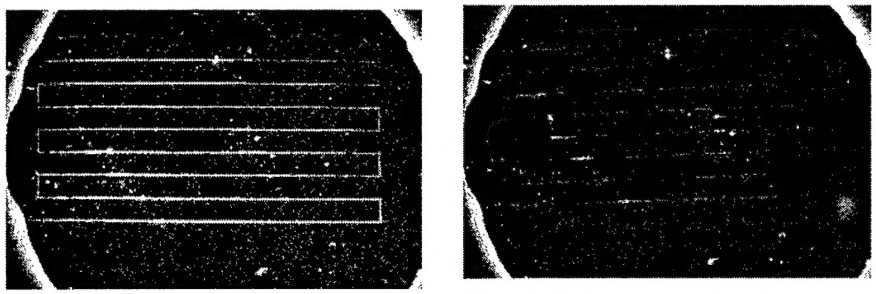


Figure 3 Left. Micrograph showing the micropattern of fluorescein on smart membrane; Right- After washing at 40°C (scale bar corresponds to 100μm).

Mesoporous Smart Hybrid Materials: We successfully grafted PNIPAAM onto mesopores of silica microspheres and demonstrated the capture and release of fluorescein molecules from the pores. Fig. 4 presents the fluorescence of dye-loaded particles as a function of time obtained from flow cytometry studies. A more rapid decrease in particle fluorescence is observed at 50°C than at 25°C, indicating that the hydrated PNIPAAm chains restrict molecular diffusion at low temperatures.

The release of rhodamine 6G dye from polymer grafted polymer particles was monitored (in situ) at 25 and 50°C by confocal microscopy. Fig. 5A presents confocal fluorescence images that show the time dependent release of rhodamine 6G at 50°C. Fluorescence profiles of the particles at various time intervals indicate (Fig. 5B) the release of the dye from the particle. Images taken at 25°C showed a much slower change in fluorescence intensity.

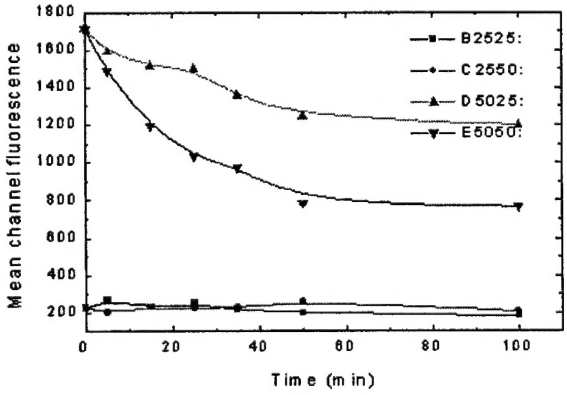


Figure 4. Release of fluorescein from PNIPAAm grafted mesoporous particles as a function of time as measured by flow cytometry. All samples were equilibrated in dye (35μM) for 2 hrs. Fluorescence of beads at 25°C was measured for beads incubated at 25°C (●) and 50°C (▲). Fluorescence of beads at 50°C was measured for beads incubated at 25°C (♦) and 50°C (▼).

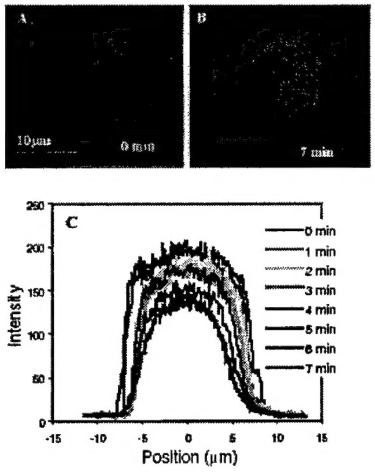


Figure 5. Confocal micrographs demonstrating the release of rhodamine 6G from the polymer grafted particles at 50°C. A. at t = 0 min, B. at t = 7 min and C. fluorescence intensity line profiles taken from the horizontal midline of the image of the particle as a function of time.

Temperature Sensitive Surfaces: NIPAAM and copolymers with *N*-tert-butylacrylamide(NTBAAm) were synthesized by ATRP technique which facilitated the successful grafting of dense and uniform polymer brushes. The thermally induced hydration transition of surface-grafted (PNIPAA) brushes was probed by surface plasmon resonance spectroscopy (SPR) (Fig.6) and contact angle measurements. The results suggest that the polymer segments in the outermost region of the brush remain highly solvated until the dilute solution lower critical solution temperature (~32°), while densely packed, less solvated segments within the brush layer undergo dehydration and collapse over a broad range of temperatures.

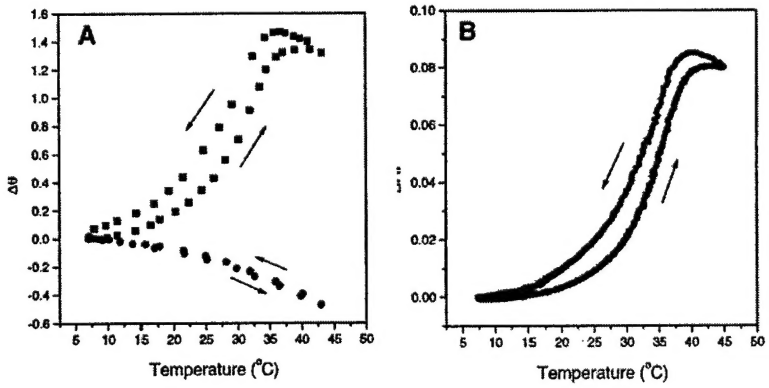


Figure 6. Temperature-dependent SPR. (A) Change in the minimum of the SPR curve ($\Delta\theta$) for the PNIPAAm layer (■) and for the control surface (a SAM formed from 11-mercapto-undecane) (●) as a function of temperature. The sample was equilibrated at every temperature for 15 min before the resonance curves were obtained. The plotted values are averages of three values. The average standard deviation in the resonance angle measurements was 0.0047 for the polymer and 0.0043 for the control. (B) Real-time measurement of change in the reflected intensity at a constant angle (74.5°) for the PNIPAAm layer as a function of temperature. The heating rate was 4.8°C/min, and the cooling rate was 4.3 °C/min.

Electrochemical Actuation of Smart Polymers: Immobilization of multi-amine elastin like polypeptides(ELPs) onto mixed self-assembled monolayers was carried out through the formation of amide bonds.

CONCLUSIONS: We successfully demonstrated that hybrid membranes comprising of silica and SRPs can function as molecular filters with reversible permeability and good stability. Micropatterning studies on hybrid membranes suggest that a wide variety of water-soluble species can be patterned without need for a specific chemisorption or affinity label using localized heating with a UV laser. Developed porous silica nanocomposite materials and modified the surface with SRPs and demonstrated that the size and surface energy of the pores can be externally and reversibly controlled to dynamically modulate the adsorption and transport of molecular species. These have potential applications in the areas of separations, controlled drug delivery, environmental remediation, bioassays, biosensing, tissue engineering.

Using a model system of silica and PNIPAAM we have demonstrated design principles and synthetic methods are applicable to a wide variety of porous structures and polymers (or small molecules) which are reversibly sensitive to different external stimuli such as temperature, light, electricity, pH, solutes, or enzymatic transformations.

SIGNIFICANCE: Silica-PNIPAAM/ELP hybrid membranes can function as molecular filters, which are sensitive to the temperature changes. These can be used as nano-valves and tunable molecular sieves. The following applications are envisaged.

1. Molecular separations through selective (tunable) ultrafiltration
2. Molecular separations through selective (tunable) chromatography
3. Molecular valves and pumps in microfluidic systems
4. Controlled drug delivery
5. Tissue engineering
6. Regeneration of bioactive surfaces

PATENT INFORMATION :

1. "Stimuli-Responsive Hybrid Materials Containing Molecular Actuators and Their Applications," G.P. Lopez, A. Chilkoti, P. Atanassov and G.V. Rama Rao, US Patent no. 6491061
2. "Stimuli-Responsive Mesoporous Materials for Control of Molecular Transport" G.P.Lopez, G.V. Rama Rao, Q.Fu, UNM-554-CIP.
3. "Stimuli-Responsive Hybrid Materials Containing Molecular Actuators and Their Applications -Control of Microbial Biofouling" G.P. Lopez, G.V. Rama Rao, and L.K. Ista, UNM-554-CIP.
4. Patent Application "Porous Microspheres that Incorporate Functional Biological Components" G.P. Lopez, J. Huang, **G.V. Rama Rao**, T. Buranda, L. Sklar and L.Ista, UNM-619, 2002.

PUBLICATIONS AND ABSTRACTS:

1. Rao, G.V.R., Fu, Q., Ista, L. K., Xu, H., Balamurugan, S., Sklar, L.A., Ward, T. L. and López, G. P. (2003) "Synthesis of smart mesoporous materials" Mat. Res.Soc. Proc. 775:7.8.1.
2. Fu, Q., Rao, G.V.R., Ista, L.K., Wu, Y., Andrzejewski, B.P., Sklar, L.A., Ward, T.L. and Lopez, G.P. (2003) "Control of Molecular Transport Through Stimuli Responsive Ordered Mesoporous Materials" *Advanced Materials* 15:1262.
3. Mendez, S., Ista, L.K., Lopez, G.P. (2003), "Use of Stimuli Responsive Polymers Grafted on Mixed Self-Assembled Monolayers To Tune Transitions in Surface Energy" *Langmuir* 19: 8115-8116.
4. Balamurugan, S., Mendez, S., Balamurugan, S.S., O'Brien II, M.J., Lopez, G.P. (2003) "Thermally Response of Poly(N-isopropyl acrylamide) Brushes Probed by Surface Plasmon Resonance" *Langmuir* 19: 2545-2549.
5. Rao, G.V.R., Krug, M.E., Balamurugan, S., Xu, H., Xu, Q. and Lopez, G.P. (2002) "Synthesis and Characterization of Silica-Poly(N-Isopropyl Acrylamide) Hybrid Membranes: Switchable Molecular Filters" *Chemistry of Materials* 14:5075.
6. Rao, G.V.R., Balamurugan, S., Meyer, D.E., Chilkoti, A., and Lopez, G.P. (2002) "Hybrid Bio-Inorganic Smart Membranes That Incorporate Protein-Based Molecular Switches" *Langmuir* 18:1819.
7. Ista, L.K., Mendez, S., Perez-Luna, V.H. and Lopez, G.P. (2001). "Synthesis of poly(N-isopropylacrylamide) on initiator-modified self-assembled monolayers" *Langmuir* 17: 2552-2555.
8. Rao,G.V.R., and Lopez, G.P. (2000) "Encapsulation of Poly (N-Isopropyl acrylamide) in Silica: A Stimuli-Responsive Porous Hybrid Material That Incorporates Molecular Nano-Valves" *Advanced Materials* 22:1692.
9. Ista, L.K., Mendez, S., and Lopez, G.P. "Attatchment and Detachment of Bacteria on Surfaces with Tunable Surface Energy" (in preparation)
10. Fu, Q. Rao,G.V.R., Lopez, G.P. "Copolymer Membranes" (in preparation)
11. Rao, G.V.R., Petsev, D., Fu, Q., Lopez, G.P. (in preparation
12. Lopez, G.P. (2003)"Stimuli-Responsive Polymers for the Dynamic Control of Interfacial Properties, **INVITED** presentation at the Army Research Office's Workshop on "Mechanics of Material Interfaces," Research Triangle Park NC, May 21-22.
13. Lopez, G.P. (2003)"Smart and Biomimetic Mesoporous Materials for Biotechnology," **INVITED** presentation at the Annual Spring Meeting of the Materials Research Society, San Francisco CA, April 24.
14. Fu, Q., Rao, G.V.R. Rao, Wu, Y., Balamurugan, S., Ward, T.L., Lopez, G.P.(2003) "Synthesis of Smart Mesoporous Materials" presented at Materials Research Society Meeting at San Francisco, CA, during April21-25.
15. Lopez, G.P. (2003)"Nanoporous Silica-Based Hybrid Membranes: Switchable Molecular Filters," **INVITED** presentation at the Nanostructures in the Environment and Technology Workshop, Albuquerque NM, January 17.

16. Lopez, G.P. (2002) "Smart Hybrid Materials: Controlling the Porosity of Silica-Based Membranes and Particles with Stimuli-Responsive Polymers," **INVITED** presentation at Duke University, Durham NC, December 3.
17. Lopez, G.P. (2002)"Nanofluidic and Biomimetic Bioanalytical Systems," **INVITED** presentation at the 49th International Symposium of the AVS, Denver CO, November 7.
18. Lopez, G.P. (2002)"Mesoporous Microbeads: Versatile Platforms for Biomolecular Assemblies," Presentation at the BioDevice Interface Science and Technology Workshop, Scottsdale AZ, Sept. 7-9.
19. Lopez, G.P. (2002) "Nanostructured and Smart Materials for BioMEMS Devices and Fluorescence Based High Throughput Screening," **INVITED** seminar presented at the University of Washington, Seattle WA, Apr. 16.
20. Rao, G.V.R., Balamurugan, S., Lopez, G.P. (2001) "Silica-Elastin Like Polypeptdie Smart Membranes-Switchable Molecular Filters" presented at American Vacuum Society's 48th International Symposium at San Francisco, California during October 28-November 2.
21. Rao, G.V.R., Ista, L.K. and Lopez, G.P.(2001) "Micropatterning on Smart Hybrid Membranes" presented at 222 American Chemical Society National Meeting at Chicago during August 26-30.
22. Rao, G.V.R., and Lopez, G.P.(2001) "Smart Porous Materials – Synthesis, Characterisation and Applications" presented at 21st CNLS conference on Principles of Soft Matter at Santa Fe, NM during May 21-25.
23. Rao, G.V.R., Fu, Q., Balamurugan, S.and Lopez, G.P.(2001) "Encapsulation of Poly (N-Isopropyl Acrylamide) in Silica: A Stimuli-Responsive Porous Hybrid Material - Molecular Nano-Valves" presented at Industrial Advisory Board Meeting for University of New Mexico and Rutgers University at Raddison Hotel, Albuquerque, NM on Feb.27.
24. Lopez, G.P. (2000)"Encapsulation of Smart Polymers in Silica: Stimuli-Responsive Porous Hybrid Materials That Incorporate Molecular Nano-valves," Presentation at the 47th International Symposium of the American Vacuum Society, Boston, Massachusetts, October 5.
25. Rao, G.V.R., and Lopez, G.P.(2000) "Encapsulation of Poly (N-Isopropyl Acrylamide) in Silica: A Stimuli-Responsive Porous Hybrid Material That Incorporates Molecular Nano-Valves" presented at Annual Symposium of American Vacuum Society-New Mexico Chapter, Albuquerque, NM during May 22-26.